Genet. Res., Camb. (1991), **57**, *pp.* 279–282 *With 2 text-figures Printed in Great Britain*

Muscle opacity (*mo*), a new mutant gene in *Xenopus laevis*, linked to the *rusty* locus

ANNE DROIN

Station de Zoologie expérimentale, Université de Genève, 154 route de Malagnou, CH-1224 Chêne-Bougeries (Switzerland)

(Received 29 August 1990 and in revised form 15 October 1990)

Summary

A new developmental mutant is described in *Xenopus laevis* (Amphibia, Anura): *muscle opacity* (*mo*). Homozygotes die at larval stage 48. The underlying defect, visible at stage 47, is a degeneration of cephalic musculature. The *mo* gene is linked to the *rusty* locus, with a map distance of 6·1 %.

1. Introduction

Linkage data are still relatively scarce in amphibian genetics. In Urodela, numerous mutant genes have been discovered in the axolotl (Armstrong, 1985); several of them have been tested for linkage but only the genes *f* (*fluid imbalance*) and *g* (*gill lethal*) have been confirmed to be linked (Humphrey, 1959); for others, linkage was suggested but not confirmed, or independent assortment was shown (Armstrong, 1984). In *Pleurodeles waltl*, a peptidase enzyme locus and two genes affecting larval and postmetamorphic skin pigmentation are linked to the sex chromosomes (Collenot *et al.* 1989).

In Anura, the first case of linkage was presented by Browder (1972) in *Rana pipiens*: a dominant *Subvital* gene (*Sbv*) is linked to the *Burnsi* locus. Since then, eight linkage groups, mainly of enzyme loci, have been established in this species and several others have been identified in other species (Wright & Richards, 1987). In *Xenopus laevis* linkage between mutant genes affecting development has not been systematically searched for, most of the genes being recessive and lethal. However, genes have been mapped to the centromere by means of diploid gynogenesis. Within the last ten years, the localization of several genes has been established (Kobel, 1981; Colombelli *et al.* 1984; Thiébaud *et al.* 1984; Reinschmidt *et al.* 1985). Moreover, with the use of isozyme markers and RFLP (restriction fragment length polymorphism), linkage groups of enzyme loci have also been mapped in this species (Graf, 1989*a*).

We present here a new recessive lethal mutant gene, *muscle opacity* (*mo*), which is linked to the pigmentation gene *rusty* (*ry*) (Uehlinger & Droin, 1969). The main characteristic of the *mo* syndrome consists of a degenerative process affecting the muscles of the head and finally leading to the death of the tadpoles. In addition to a description of the *mo* phenotype, linkage data are reported.

2. Material and Methods

Laboratory-bred as well as imported adult *Xenopus laevis laevis* were used in the crosses. Egg laying, fertilization, and rearing of tadpoles were according to standard methods used in our laboratory (Droin & Chavane, 1976). Developmental stages (st.) are numbered according to Nieuwkoop and Faber (1956). For histological analysis, tadpoles were fixed in Zenker's or Bouin's fluids, serially cut at 6 μm and stained with haemalum-eosin.

3. Results and Discussion

(i) *Phenotype*

Mutant embryos and tadpoles develop normally up to the 9th day (st. 47). At this stage, mutant tadpoles can be distinguished from normal sibs by a characteristic opacity of the ventral muscles of the head. This anomaly seems more conspicuous in tadpoles, homozygous for the *rusty* mutant gene; presumably this is an effect of the reddish tinge of their skin, apt to improve visibility under incident light. The tinge is due to the persistence of egg pigment into larval stage. No other abnormality has been found in *rusty* (Uehlinger *et al.* 1971).

Muscle opacity in *mo* affects particularly the large interhyoideus muscle extending transversely throughout the ventral part of the head; it is more pronounced in the anterior part of the muscle. Two other muscles

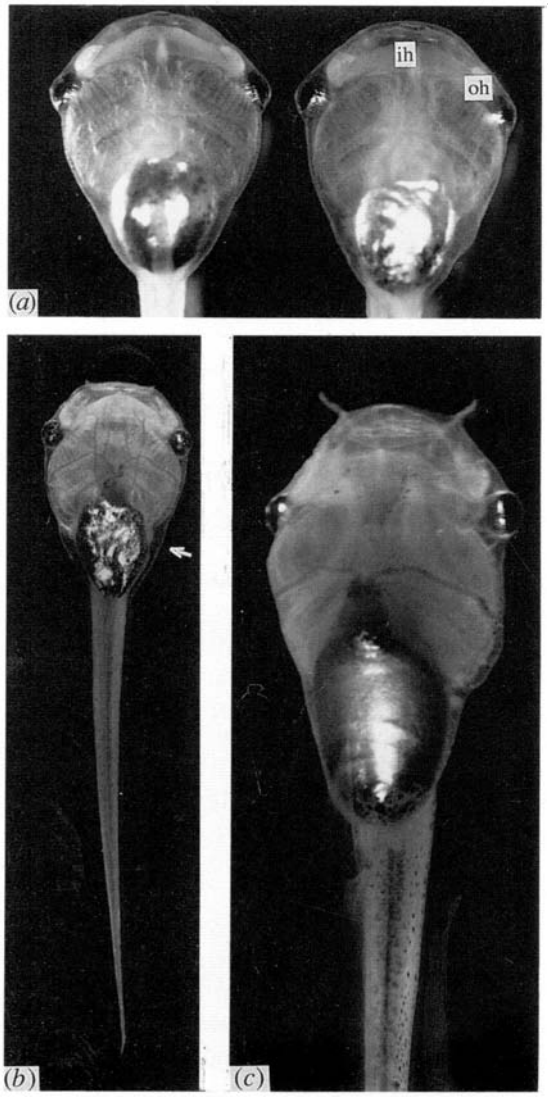

Fig. 1. (*a*) Ventral view of a *mo ry* mutant (left) and of a normal *ry* tadpole (right) aged 10 days (st. 48). The opacity of the rostral part of the interhyoideus muscle (ih) and of the orbito-hyoideus muscles (oh) of the *mo* tadpole is clearly visible. × 10·5. (*b*) A *mo* tadpole aged 20 days arrested at st. 48. (*c*) A normal tadpole of the same age and at the same magnification. Note the small size of the mutant and the marbled aspect of the iridophore layer of the belly (arrow). × 7·2.

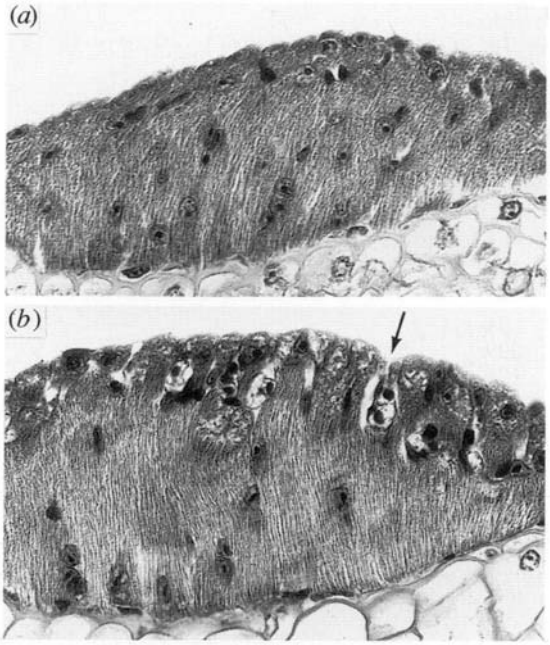

Fig. 2. Longitudinal sections of the orbito-hyoideus muscles of two mutants. At 11 days (*a*), the bundle structure is still very similar to that of a normal muscle, while at 23 days (*b*), blood infiltration (arrow) and progressive degeneration of the fibres are observed. × 320.

of the hyoid arch are also opaque, the thick lateral symmetrical muscles situated ventrally to the eyes, m. orbito-hyoideus or levator hyoideus (Fig. 1*a*). In more advanced stages, opacity can affect three other muscles, namely the two other symmetric ones of the hyoid arch, m. quadrato-hyoangularis or depressor mandibulae and the small transverse muscle situated rostrally to the heart, m. transversus ventralis. Sections through these muscles reveal that the opaqueness is due to degeneration of the muscles. The periphery of their bundles is disorganized, with loose cytoplasm and blood infiltration, becoming more pronounced as time proceeds (Fig. 2). This deterioration affects also the fibres themselves which lose their striation. By contrast, the entire musculature of the rump and tail regions seems hardly affected, and heart muscle is normal.

Evolution of the syndrome is slow; tadpoles feed normally. At fifteen days, other abnormalities typical of an arrest of growth are observed, probably due to the fact that the muscles are no longer functional and prevent the tadpoles from feeding. When normal tadpoles have reached st. 49, the mutants are blocked at st. 48; their head and body are smaller and narrower, the gut slender with a marbled aspect of the iridophore layer (Fig. 1*b*, *c*), and the thymus and limb buds less developed. The heart, blood vessels and pronephros become red due to a transient accumulation of blood. In the following days, the phenotype presents the typical aspect of general degeneration, the tadpoles become blind, the gut very thin with a protruding gall bladder and distended pro- and mesonephros tubuli. The mutant tadpoles more and more resemble the various lethal dwarf mutants (Droin, 1974, 1988). They begin to die around the 20th day.

This mutation is the third one found in our laboratory involved with the development of muscles of young tadpoles of *Xenopus*. The mutant phenotype *folded jaw* was found to be due to a morphological deficiency of the m. quadrato-hyoangularis, which is not long enough to be inserted on the cartilage of the mandibular arch (Droin *et al.* 1968). In the *immobile* mutant, a defect in the function of the muscles prevents them from contracting (Droin & Beauchemin, 1975). In the *mo* mutant, the muscles are well developed and functioning, but then degenerate. Such

Table 1. *Segregation from seven crosses* $\frac{ry\ mo}{+\ +} \times \frac{ry\ mo}{+\ +}$

No. of cross	Phenotypes and number of tadpoles					Recombinants	
	Total	Normal	*ry mo*	*ry*	*mo*	Total	Percentage
1	87	58	20	7	2	9/87	10·3 ± 3·5
2	169	125	37	3	4	7/169	4·1 ± 1·6
3	108	76	29	1	2	3/108	2·8 ± 1·8
4	45	31	12	1	1	2/45	4·4 ± 3·2
5	34	26	6	1	1	2/34	5·9 ± 4·2
6	229	169	50	5	5	10/229	4·4 ± 1·4
7	181	124	38	13	6	19/181	10·5 ± 2·4
	853	609	192	31	21	52/853	6·1 ± 0·9

mutations illustrate the complex mechanisms of gene action intervening at different levels of muscle differentiation. It should be possible, by chimaeric combinations, to determine whether *mo* selectively acts upon head musculature in particular, or whether postcranial musculature appears unaffected only because of differential maturation along the body axis.

(ii) *Genetics*

The *rusty* mutation, which is inherited as a recessive mendelian factor with a constant expression, was found in the offspring of two families, one parent of which came from a nuclear transplantation egg. Its origin was not determined (Uehlinger & Droin, 1969). Since then the *rusty* mutation, which can be useful as a marker in embryological experimentation, has been maintained through several generations in hetero- and homozygous stocks. The *mo* mutation was found in the F_2 of a family created by a mating between a homozygous *rusty* female (♀ 30) and a ♂ (♂ 34) descended also from an adult ♂ issued from a nuclear transfer. As the mutant tadpoles were identified only after the death of their parents and grandparents, backcrosses were impossible; the origin of this mutation cannot be determined either.

When mutant tadpoles of the first F_2 mating were observed (obtained from a cross between two F_1 individuals), it was obvious that nearly all the *rusty* tadpoles presented the abnormal phenotype while only a few non-*rusty* tadpoles showed the same abnormalities. The data of the different matings are summarized in Table 1. They show that segregation deviates from independence in a two-factor cross (9:3:3:1), thus demonstrating linkage between *mo* and *rusty*. In these matings between heterozygotes for the two mutations (*ry*/+ and *mo*/+), in addition to the double-recessive mutants (192/853) obtained, there was a small number of either *rusty* or *mo* single-recessive mutants. The number of recombinants – 52 tadpoles out of 853 examined in 7 different matings – corresponds to 6·1% of recombination. From these data we conclude that the two mutations were linked in coupling phase.

Since the terminal phenotype of the *mo* mutant is similar to that of various *dwarf* mutants, matings were performed between individuals, heterozygous for *mo* and the four *dwarf* genes of our mutant collection. No mutant segregant was found, showing that *mo* and the different *dwarf* mutations are not allelic.

Recently, two developmental mutations have been attributed to different linkage groups, periodic albinism (a^p) to group I (Graf, 1989*a*) and polydactyly (*pd*) to group III (Graf, 1989*b*). Analysis by interspecies hybridization involving isozyme markers might allow us to assign *mo* and *ry* to one of the known linkage groups.

I am grateful to Professor H. Gloor for critically reading the manuscript. I thank Mrs M. Quentin for technical assistance, MM. A. Solaro and A. Portianucha for the photographs and Mrs Y. Develey for typing assistance. This work was supported by the University of Geneva.

References

Armstrong, J. B. (1984). Genetic mapping in the Mexican axolotl, *Ambystoma mexicanum*. *Canadian Journal of Genetics and Cytology* **26**, 1–6.

Armstrong, J. B. (1985). The axolotl mutants. *Developmental Genetics* **6**, 1–25.

Browder, L. W. (1972). A subvital gene in *Rana pipiens* linked to the Burnsi locus. *Genetical Research* **20**, 263–268.

Collenot, A., Dournon, C. & Lauthier, M. (1989). Genetic evidence for linkage with the Z and W sex chromosomes of two distinct couples of alleles controlling larval and post-metamorphic skin pigmentation in salamander. *Biology of the Cell* **67**, 1–7.

Colombelli, B., Thiébaud, Ch. H. & Müller, W. P. (1984). Production of WW super females by diploid gynogenesis in *Xenopus laevis*. *Molecular and General Genetics* **194**, 57–59.

Droin, A. (1974). Trois mutations récessives létales, *dwarf*-I (*dw*-I), *dwarf*-II (*dw*-II) et *precocious oedema* (*p.oe*) affectant les têtards de *Xenopus laevis*. *Annales d'Embryologie et de Morphogenèse* **7**, 141–150.

Droin, A. (1988). Three new analogous mutations in *Xenopus laevis*. *Acta Embryologiae et Morphologiae experimentalis*. (New series) **9**, 115–127.

Droin, A. & Beauchemin, M. L. (1975). *Immobile* (*im*), a recessive lethal mutation of *Xenopus laevis* tadpoles. *Journal of Embryology and Experimental Morphology* **34**, 435–449.

Droin, A. & Chavane, M. C. (1976). A recessive semi-lethal mutation, *distended lungs* (*dl*) affecting the tadpoles of *Xenopus laevis*. *Acta Embryologiae Experimentalis* **3**, 273–279.

Droin, A., Reynaud, J. & Uehlinger, V. (1968). *Folded jaw* (*fj*), une mutation létale récessive affectant le développement de la mâchoire chez *Xenopus laevis*. *Revue Suisse de Zoologie* **75**, 531–538.

Graf, J. D. (1989*a*). Genetic mapping in *Xenopus laevis*: eight linkage groups established. *Genetics* **123**, 389–398.

Graf, J. D. (1989*a*). Genetic mapping of a mutation causing *polydactyly* (*pd*) in *Xenopus laevis*. *Experientia* **45**, A64 (Abstract).

Humphrey, R. R. (1959). A linked gene determining the lethality usually accompanying a hereditary fluid imbalance in the Mexican axolotl. *The Journal of Heredity* **50**, 279–286.

Kobel, H. R. (1981). Gene mapping in *Xenopus* (Anura, Pipidae). *Monitore zoologico italiano* **17**, 109–118.

Nieuwkoop, P. D. & Faber, J. (1956). *Normal Table of Xenopus laevis* (*Daudin*) Amsterdam: North-Holland.

Reinschmidt, D., Friedman, J., Hauth, J., Ratner, E., Cohen, M., Miller, M., Krotoski, D. & Tompkins, R. (1985). Gene-centromere mapping in *Xenopus laevis*. *The Journal of Heredity* **76**, 345–347.

Thiébaud, Ch. H., Colombelli, B. & Müller, W. P. (1984). Diploid gynogenesis in *Xenopus laevis* and the localization with respect to the centromere of the gene for periodic albinism a^p. *Journal of Embryology and Experimental Morphology* **83**, 33–42.

Uehlinger, V., Beauchemin, M.-L. & Droin, A. (1971). The behaviour of the egg pigment in wild-type and *rusty* tadpoles of *Xenopus laevis*. *Journal of Embryology and Experimental Morphology* **26**, 571–585.

Uehlinger, V. & Droin, A. (1969). Origine et hérédité de la mutation *rusty* (*ry*) du batracien anoure *Xenopus laevis* (Daudin). *Archiv der Julius Klaus-Stiftung* **43/44**, 48–54.

Wright, D. A. & Richards, C. M. (1987). Linkage groups in the leopard frog, *Rana pipiens*. In *Genetic Maps* (ed. S. J. O'Brien), vol. 4, pp. 417–421. Cold Spring Harbor Laboratory.